# The Role and Molecular Mechanism of Endoplasmic Reticulum Stress in Cardiomyocyte Apoptosis

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**Abstract.** Endoplasmic reticulum stress (ERS) is a protein folding imbalance state caused by ischemia/metabolic abnormalities, which triggers programmed cell death (apoptosis) by activating the PERK/IRE1/ATF6 CHOp pathway. ERS and apoptosis participate in the cell injury process of cardiovascular diseases such as myocardial infarction and heart failure. Targeting ERS-apoptosis axis has become a new therapeutic strategy. This paper aims to explore the role and molecular mechanism of ERS in cardiomyocyte apoptosis, and to introduce the therapeutic direction of cardiovascular diseases derived from the intervention of different stages of ERS in molecular mechanism and its association with diseases. The process of ERS-induced cardiomyocyte apoptosis is related to some diseases, such as myocardial infarction, heart failure and so on. Therefore, several therapeutic strategies have been explored based on these diseases and molecular mechanisms. It is mainly divided into two stages of intervention. During the compensatory period, the UPR protective pathway is enhanced to improve cardiac strain and inhibit early PERK overactivation. Gene silencing strategies and small molecule inhibitors derived from CHOP pathway targeted therapy in apoptotic intervention. In addition, this paper also puts forward some future directions for the existing treatment strategies, such as multi-pathway synergistic intervention and spatio-temporal specific regulation.

**Keywords:** Endoplasmic reticulum stress, cardiomyocyte apoptosis, cardiovascular protection.

## 1. Introduction

Apoptosis, which maintains homeostasis and eliminates abnormal cells, is a programmed cell death process regulated by genes. The chemical features of apoptosis include cell contraction, chromatin condensation, and membrane disruption, leading to the formation of apoptotic bodies, with biochemical markers: DNA fragmentation forming a ladder pattern of 180 BP, phosphatidylserine (PS) exposed. And activation of caspase enzyme such as caspase-3, and no inflammatory reaction such as phagocytosis of apoptotic cells by macrophage or adjacent cells, and no leakage of contents Triggered via two core pathways—the extrinsic (death receptor-mediated) and intrinsic (mitochondrial) pathways—apoptosis culminates in caspase-3 activation and plays pivotal roles in development, immunity, and diseases (e.g., cancer, myocardial infarction). The endoplasmic reticulum (ER) is an important intracellular organelle that plays a crucial role in maintaining cellular homeostasis. When the body is subjected to adverse external stimuli, it can induce endoplasmic reticulum stress (ERS) and unfolded protein response (UPR). As a quality control organelle that maintains protein homeostasis, its protein quality control system includes endoplasmic reticulum-related degradation, protein chaperones, and autophagy. When the protein homeostasis is disrupted and unfolded or misfolded proteins accumulate in the ER, ERS is activated[1]. Proteostasis is an important basis for cell survival. Once protein homeostasis is out of balance, it will lead to a variety of diseases, including cardiovascular diseases, cancer diseases and so on[2]. ER is a control organelle that controls the quality of the proteins it produces, allowing only normal proteins to be released from its vesicles[3]. Protein quality control system maintains protein homeostasis through molecular chaperones (such as BiP, Grp94), ATPase and two major degradation systems (ubiquitin-proteasome and autophagy-lysosome pathways)[4]. The ER cleans misfolded proteins produced under stress through a folding mechanism, transports them retrogradely to the cytosol, and completes ubiquitin-proteasome-dependent degradation through the ERAD pathway[5,6]. However, persistent misfolded proteins can accumulate and trigger ERS, this process activates the UPR in an adaptive response to endoplasmic reticulum stress. ERS is critical for cardiomyocyte apoptosis, which is the common

pathological mechanism of many cardiovascular diseases. The accumulation of incorrectly folded proteins or the imbalance of calcium homeostasis in the endoplasmic reticulum of cardiomyocytes is the fundamental reason for triggering the unfolded protein response (UPR), and apoptosis is initiated through specific signaling pathways if stress persists. ERS induces cardiomyocyte apoptosis through two core pathways, PERK/CHOP and IRE1α/JNK and cross-amplifies with the mitochondrial apoptosis pathway.

Interventions targeting different stages of endoplasmic reticulum stress may become a new strategy for cardiovascular protection, and significant progress has been made in this field of cardiovascular protection research both domestically and internationally. For example, during the compensatory phase, interventions have been made by enhancing the protective pathway of UPR. The chemical partner 4-phenylbutyric acid (4-PBA) has been developed, which is an FDA-approved short-chain fatty acid that can reduce the expression of GRP78/CHOP in myocardial ischemia-reperfusion (I/R) models. The novel taurodeoxycholic acid (TUDCA) nanoparticles developed by Shanghai Jiao Tong University (targeting the myocardial mitochondrial endoplasmic reticulum contact point) significantly improved the survival rate of mice after I/R. There are many cutting-edge breakthroughs and in-depth research directions in the field of cardiovascular protection for intervention strategies targeting different stages of endoplasmic reticulum stress (ERS), such as spatiotemporal specific regulation, multi-path intervention, organoid and artificial intelligence prediction, etc. The UPR dual regulator developed by GlaxoSmithKline (GSK), which simultaneously inhibits IRE1α ribonuclease and activates ATF6, has entered the preclinical stage. The Cambridge University team used cardiac organoids to screen for ERS inhibitors, and an artificial intelligence model predicted new CHOP-DNA binding sites. In the Stanford University experiment, the light-controlled UPR activation system (such as photosensitive PERK inhibitors) achieved precise intervention.

This paper starts with the factors that lead to endoplasmic reticulum stress, and then elaborates on the process and molecular role of apoptosis and endoplasmic reticulum stress-induced cardiomyocyte apoptosis. At the same time, in view of the association between the above molecular mechanisms and processes and diseases, some therapeutic directions and emerging targets are proposed. Finally, some new strategies for the treatment of cardiovascular diseases and related scientific research achievements are proposed.

## 2. Triggers of Endoplasmic Reticulum Stress

The triggering of ERS is directly related to various pathological stimuli received by myocardial cells. When myocardial cells encounter metabolic abnormalities such as ischemia, oxidative stress, calcium overload or hyperglycemia, activation of the UPR results from the accumulation of unfolded/misfolded proteins in the ER, and the imbalance of calcium homeostasis is also a core factor in inducing ERS. This process involves two key events, one of which is the dissociation of the chaperone protein GRP78 (BiP). Under normal conditions, GRP78 binds to ERS sensors such as IRE1α, PERK and ATF6 to maintain its inactive state. When unfolded proteins accumulate, GRP78 preferentially binds to these abnormal proteins, leading to sensor release and activation. The second is the disruption of calcium homeostasis. Dysfunction of the sarcoplasmic reticulum calcium pump (SERCA) or calcium leakage can lead to a decrease in calcium concentration in the endoplasmic reticulum, further weakening the protein folding ability of the ER and exacerbating the progression of ERS.

## 3. Apoptosis and Molecular Mechanism

### 3.1. The Definition of Apoptosis and Associated Role of Autophagy

Apoptosis is a programmed cell death process precisely regulated by genes, characterized by cell contraction, chromatin condensation and apoptotic body formation, which is carried out through the

Caspase cascade activated by death receptors or mitochondrial pathways. ERS is an important mechanism of inducing apoptosis. When ERS occurs, three transmembrane protein receptors on the endoplasmic reticulum membrane are activated and the apoptotic program is initiated. Autophagy maintains cellular homeostasis by degrading and recycling biomolecules and damaged organelles, a highly conserved process in eukaryotes. Autophagy can be divided into three categories: macroautophagy, microautophagy and chaperone-mediated autophagy. ERS usually activates the non-selective macroautophagic pathway; Both of them are cytoprotective mechanisms, which maintain physiological homeostasis by eliminating abnormal intracellular components. Therefore, autophagy can serve as an alternative pathway to ERAD, participating in the clearance of excess proteins within the endoplasmic reticulum. In endoplasmic reticulum stress (ERS) -induced autophagy, the unfolded protein response (UPR) regulates the initiation and progression of autophagy through its three core branch signaling pathways (PERK, IRE1α, ATF6)[7].

### 3.2. Molecular Mechanism

UPR achieves the transition from stress compensation to pro-apoptosis through three pathways: IRE1 α - XBP1, PERK-eIF2 α - ATF4 CHOP, and ATF6. IRE1 α, as a transmembrane protein with dual protein kinase and ribonuclease activities, undergoes dimerization, oligomerization and trans phosphorylation upon activation[8]. Its nuclease activity can cleave the 26nt intron of XBP1 mRNA to generate XBP1s, which enhance protein folding and degradation ability by upregulating partner proteins such as GRP78 and PDI, as well as ERAD components[9]. If stress persists, IRE1 α recruits TRAF2 to activate the ASK1-JNK pathway, releases Bax/Bak through phosphorylation of Bcl-2, and activates c-Jun mediated transcription of apoptotic genes. PERK activation regulates eIF2α through dimerization and autophosphorylation, inhibits overall protein synthesis to reduce endoplasmic reticulum burden, and selectively translates ATF4 mRNA. ATF4 maintains redox balance by upregulating antioxidant genes. The sustained expression of ATF4 activates CHOP, promoting Bax/Bak oligomerization and mitochondrial membrane permeability by downregulating Bcl-2 and upregulating Bim/Puma, and activating ERO1 α to exacerbate oxidative stress. Under stress conditions, ATF6, a transmembrane protein, is transported to the Golgi apparatus, where it is subsequently cleaved by the S1P/S2P protease. The released cytoplasmic fragment ATF6f enters the nucleus, such as GRP78 and XBP1, and functions to regulate the UPR gene. Under sustained stress, it synergistically upregulates death receptor 5 with CHOP, activating the exogenous apoptotic pathway.

When UPR fails to restore ER homeostasis, it directly triggers cell death through an ER-specific apoptotic pathway: Caspase-12 (Caspase-4/5 in humans), as a key effector molecule on the ER membrane, activates calpain through calcium signaling mediated by the CHOP-ERO1α pathway, and then translocates from the ER membrane to the cytoplasm. It directly activates caspase-9 (independent of mitochondrial cytochrome C release) and ultimately executes apoptosis via caspase[10]. The mitochondrial apoptosis pathway undergoes cross-activation during this process. CHOP and JNK promote Bax/Bak oligomerization and insert into the mitochondrial membrane to form pores, releasing cytochrome C to activate caspase-9/3. At the same time, calcium released from the endoplasmic reticulum enters the mitochondria through the mitochondrial calcium uniporter, inducing mitochondrial membrane potential collapse. The endoplasmic reticulum mitochondrial contact points (MAMs), as structural regions rich in IP3R, VDAC1, and GRP75, promote mitochondrial apoptosis by accumulating Bax/Bak under stress. The calcium released by the endoplasmic reticulum enters the mitochondria through the IP3R-GRP75-VDAC1 channel, further exacerbating oxidative stress and promoting the process of apoptosis.

Ferroptosis is characterized by high expression of unsaturated fatty acids catalyzed by iron ions or lipoxygenases in the cell membrane, resulting in lipid peroxidation, and reduced activity of the core regulatory enzyme glutathione peroxidase 4 (GPX4) in the antioxidant system, triggering cell death, a mode of cell death distinct from apoptosis and autophagy. Iron death is mainly caused by the increase of iron iron-positive divalent ion concentration in cells. Nuclear factor E2-related factor 2

(Nrf2) plays a key role in the regulation of ferroptosis, regulating intracellular iron metabolism. Currently, many studies have shown that ferroptosis induced by endoplasmic reticulum stress (ERS) leads to $Fe^{2+}$ accumulation and the formation of lipid peroxidation through the PERK-Nrf2-HO-1 pathway. ATF4 can increase the expression level of Xc through transcriptional regulation, thereby inhibiting the occurrence of ferroptosis. Recent experimental data suggest that there are differences in the patterns of cell death mediated by the three UPR pathways[7]. Recent experimental data suggest that there are differences in the patterns of cell death mediated by the three UPR pathways.

The main features of Pyroptosis include nuclear condensation, random fragmentation and degradation of chromatin DNA, and the formation of numerous pores within the cell membrane, which stimulates an inflammatory response that leads to cell swelling and rupture, a pro-inflammatory cell necrosis based on the pore-forming activity of the Gasdermin protein family[11]. The recruitment of inactive pro-caspase-1 is the main mechanism of Pyroptosis. Upon sensing an external stimulus, the multiprotein complex inflammasome cleaves pro-caspase-1 into active caspase-1. Caspase-1 has the function of cleaving and activating GSDMD protein to N-GSDMDS, which can bind to the cell membrane to form 1 – 2 nanometer-sized pores, leading to the outflow of cell contents by destroying the integrity of the cell membrane, causing changes in osmotic pressure, and ultimately leading to cell swelling and rupture[12]. Caspase-1 cleaves inactive pro-IL-1β and pro-IL-18 to produce mature cytokines IL-1β and IL-18 with pro-inflammatory activity, and then amplifies inflammatory signaling pathways[13].

The final stage of apoptosis is driven by a series of ordered molecular events, ultimately achieving programmed clearance of cells. Activated caspase-3 cleaves the inhibitory CAD protein (ICAD) to release CAD nuclease, triggering DNA fragmentation and completing genome fragmentation. At the same time, caspase-3 activates a cascade, promoting membrane eversion of phosphatidylserine (PS) and providing specific recognition signals for apoptotic cells. On this basis, cells contract and form membrane-bound apoptotic bodies, which are ultimately recognized and engulfed by macrophages, ensuring that there is no leakage of cellular contents during the apoptosis process and avoiding inflammatory reactions.

## 4. Association with Disease

### 4.1. Myocardial Infarction and Heart Failure

The association of myocardial infarction (MI) and heart failure (HF) with endoplasmic reticulum stress (ERS) represents a vicious cycle that is triggered by ischemic injury, mediated by ERS, and ultimately leads to deterioration of cardiac function. In the process of myocardial infarction, myocardial ischemia and hypoxia caused by acute coronary artery occlusion directly destroy the homeostasis of endoplasmic reticulum, which is manifested by unfolded protein aggregation, calcium homeostasis disorder (SERCA2a dysfunction) and oxidative stress, and then activate the three core pathways of UPR (PERK, IRE1α, ATF6). The PERK-CHop axis drives cardiomyocyte apoptosis by inhibiting the anti-apoptotic protein Bcl-2 and upregulating the pro-apoptotic factor Bim/Puma, while the IRE1α-JNK pathway aggravates mitochondrial dysfunction, leading to the expansion of infarct size.

In addition, ERS-induced inflammatory responses (e.g, NLRP3 inflammasome activation) and ROS overproduction accelerate myocardial injury and promote fibroblast activation to drive ventricular fibrotic remodeling. During the development of heart failure, persistent ERS (such as caused by pressure overload or metabolic abnormalities) aggravates myocardial cell loss and interstitial collagen deposition through the CHOP-dependent apoptosis pathway and ATF6-mediated fibrosis signal, and further impairs cardiac systolic/diastolic function. At the same time, ERS interferes with the expression of calcium regulatory proteins (such as RyR2, SERCA2a), induces arrhythmia, and forms a positive feedback cycle of ERS-cardiac function deterioration-ERS aggravation.

### 4.2. Diabetic Cardiomyopathy

Diabetic cardiomyopathy (DCM) is closely associated with endoplasmic reticulum stress (ERS), which is activated by chronic hyperglycemia and lipotoxicity through multiple mechanisms leading to cardiomyocyte apoptosis, fibrosis and cardiac dysfunction. Endoplasmic Reticulum (ER) dysfunction in cardiomyocytes under high glucose conditions is caused by protein synthesis overload, oxidative stress (NADPH oxidase activation) and accumulation of advanced glycation end products (AGEs), triggering the PERK-eIF2α-ATF4-CHop pathway. Promote CHOP protein to up-regulate pro-apoptotic factors (such as Bax/Bak) and inhibit anti-apoptotic proteins (such as Bcl-2), and accelerate the loss of myocardial cells. At the same time, dysregulation of the IRE1α-XBP1 pathway further exacerbates the inflammatory response (TNF-α/IL-6 release) and insulin resistance (IRS-1 serine phosphorylation), whereas aberrant activation of the ATF6 pathway promotes myocardial fibrosis (TGF-β1/collagen deposition). In addition, ERS-induced abnormalities in calcium regulation, such as decreased SERCA2a activity and RyR2 calcium leakage, directly impair myocardial contractility and form a vicious circle with mitochondrial dysfunction (ROS burst) to promote DCM progression. Targeting ERs (such as SGLT2 inhibitors, chemical chaperones TUDCA, or CHOP gene silencing) can be an important strategy to improve diabetic cardiomyopathy by restoring protein homeostasis and reducing apoptosis.

## 5. Therapeutic Targets and Research Progress

Interventions aimed at different stages of endoplasmic reticulum stress can become a new strategy for cardiovascular protection. Because of this exploration, remarkable achievements have been made at home and abroad. The current scientific research achievements and immunotherapy are described in the following two stages: compensatory stage (UPR activation stage) and apoptotic stage (ERS irreversible stage).

### 5.1. Compensatory Intervention

Among the chemical partners that target ER stress, the most extensively studied internationally are 4-PBA and TUDCA. As a short-chain fatty acid approved by FDA, 4-PBA has been proven to significantly reduce the expression levels of GRP78 and CHOP in the myocardial ischemia-reperfusion (I/R) model. Taurine Ursodeoxycholic Acid (TUDCA) has been shown to improve myocardial strain in heart failure patients in a clinical trial in the United States by stabilizing GRP78 and inhibiting the PERK pathway. In terms of domestic research progress, Shanghai Jiao Tong University has developed a new type of TUDCA nanoparticle (targeting the myocardial mitochondrial endoplasmic reticulum contact point), which significantly improves the survival rate of mice after I/R (Previously, ligustrazine enhance UPR compensation by upregulating XBP1s has been found by the team of the Chinese Academy of Sciences. In terms of regulating the core sensors of UPR, interventions targeting the IRE1 α - XBP1 pathway have led to gene therapy AAV9-XBP1s internationally, which reduced apoptosis by 50% in a pig myocardial infarction model. Domestically, Nanjing Medical University discovered that salvianolic acid selectively activates XBP1 splicing through the IRE1 α ribonuclease inhibitory domain (conserved site Arg893). The ATF6f (truncated) adenovirus vector designed by the Peking University team significantly improved pressure overload-induced myocardial hypertrophy in response to the ATF6 pathway. In terms of inhibiting early PERK overactivation, international studies have shown that the PERK inhibitor GSK2606414 is limited by liver toxicity, and the new generation of allosteric inhibitors is optimizing safety. The Chinese Academy of Medical Sciences found that berberine alleviates endoplasmic reticulum stress by inhibiting the eIF2α α/ATF4/CHOP axis, and has no hepatotoxicity.

### 5.2. Apoptosis Intervention

In the field of CHOP pathway targeted therapy, international research progress includes the completion of phase I clinical trials of siRNA CHOP liposomes (NCT04853199), which significantly

reduce inflammatory factors after myocardial infarction, and the entry of TRB3-CHOP interaction inhibitor NSC139021 into preclinical studies. At the same time, domestic teams have also made breakthroughs. Fudan University has developed a miR-181a-5p exosome delivery system targeting CHOP3'UTR (reducing fibrosis by 30% in a pig model), and Shanghai Institute of Materia Medica has found that ginsenoside Rg3 exerts anti-apoptotic effects by blocking CHOP/DR5 binding.

In terms of Caspase-12/4/5 inhibition research, international progress has shown that the caspase-4 inhibitor DSB120 has a clear protective effect on sepsis-related cardiomyopathy (not yet extended to cardiovascular indications). The Zhejiang University team discovered that baicalin inhibits the calpain/caspase-12 pathway by chelating calcium ions, providing a new strategy for cardiovascular protection.

In the field of regulating endoplasmic reticulum mitochondrial couplings (MAMs), significant progress has been made in international research. The IP3R inhibitor Xestospongin B can maintain calcium homeostasis in heart failure models, and the MCU blocker Ruthenium 360 has entered phase II clinical trials. At the same time, domestic teams have also made breakthroughs. Resveratrol reduces calcium overload through SIRT3-mediated VDAC1 deacetylation, and the MAMs stabilizing peptide TAT-MAM7 developed by Wuhan University significantly inhibits cardiomyocyte apoptosis.

Overall, international research focuses more on precise target interventions and gene therapy technologies, while China has unique advantages in the development of natural medicines and the integration of traditional Chinese and Western medicine. In the future, it is necessary to overcome delivery system bottlenecks (such as cardiac target exosomes) and strengthen cross-center clinical validation. ERS intervention has shifted from single pathway inhibition to dynamic network regulation, providing a new therapeutic paradigm for cardiovascular diseases.

## 6. Conclusion

Cardiomyocyte apoptosis induced by endoplasmic reticulum stress is a process in which endoplasmic reticulum stress activates the caspase cascade through PERK-CHop and IRE1α-JNK pathways to drive programmed apoptosis of cardiomyocytes, both of which aggravate cardiovascular injury. This article focuses on the molecular mechanism and related processes of endoplasmic reticulum stress-induced cardiomyocyte apoptosis, followed by its association with disease. Endoplasmic reticulum stress activates the PERK-CHop and IRE1α-JNK pathways, triggers the caspase cascade, induces cardiomyocyte apoptosis, and promotes the development of cardiovascular diseases. The intervention of endoplasmic reticulum stress at different stages can be a powerful new strategy for cardiovascular protection. Cardiovascular protection can be achieved by intervention and regulation of UPR core sensors in two different stages, namely, the compensatory stage and apoptotic stage, and inhibition of early PERK over-activation. At the same time, it puts forward the breakthrough and future direction of spatiotemporal specific regulation, such as light-controlled UPR activation system (such as photosensitive PERK inhibitor) and multi-pathway synergistic intervention. The treatment of cardiovascular disease through the intervention of different stages of endoplasmic reticulum stress is an emerging cardiovascular protective strategy. This paper proposes and elaborates on this strategy and proposes the direction of future progress. Although targeting early (e.g, enhanced UPR compensation) and late (e.g, inhibition of CHOP) ERS pathways has demonstrated potential for cardiovascular protection, its clinical application still faces key challenges such as insufficient targeting specificity, difficulty in balancing compensatory and apoptotic signaling, and uncertain long-term safety. Precise interventions targeting different stages of endoplasmic reticulum stress (ERS), including enhancing early UPR compensation (such as XBP1 activation) and blocking late apoptotic signaling (such as CHOP inhibition), are expected to become breakthrough strategies for cardiovascular disease treatment through new drug delivery systems and gene editing technologies, but key issues such as spatiotemporally specific regulation and individualized treatment still need to be addressed.

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